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LACK OF SOYBEAN ROOT ELONGATION RESPONSES TO MICROMOLAR MAGNESIUM ADDITIONS AND FATE OF ROOT-EXUDED CITRATE IN ACID SUBSOILS

Yohey Hashimoto [a]; T. Jot Smyth [a]; Daniel W. Israel [b];Dean Hesterberg [a]
[a] Department of Soil Science, North Carolina State University, Raleigh, North Carolina, USA [b] USDA-ARS, Department of Soil Science, North Carolina State University, Raleigh, North Carolina, USA

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LACK OF SOYBEAN ROOT ELONGATION RESPONSES TO MICROMOLAR MAGNESIUM ADDITIONS AND FATE OF ROOT-EXUDED CITRATE IN ACID SUBSOILS

Yohey Hashimoto and T. Jot Smyth □ *Department of Soil Science, North Carolina State University, Raleigh, North Carolina, USA*

Daniel W. Israel □ *USDA-ARS, Department of Soil Science, North Carolina State University, Raleigh, North Carolina, USA*

Dean Hesterberg □ *Department of Soil Science, North Carolina State University, Raleigh, North Carolina, USA*

□ *Additions of micromolar concentrations of magnesium (Mg) to hydroponics enhance aluminum (Al) tolerance of soybean by increasing citrate secretion from roots and external complexation of toxic Al species. The objective of this study was to assess the ameliorative effect of Mg additions on soybean root elongation into mineralogically different acid soils. Roots of soybean seedlings grew for 28 days into acid soils treated with three Mg levels in their soil solution (Control, 150 and 300 μM) and lime. Root growth in the acid soils and aboveground dry matter responses to the Mg treatments were less than for the lime treatments. Citrate fate in the acid soils revealed that 66–99% of added citrate was either adsorbed or biodegraded, suggesting that root secreting citrate in the soil abundant with Al and iron (Fe) hydroxides potentially reduces the availability to complex rhizotoxic Al. A calcium (Ca) deficiency may have constrained root growth response to the Mg-treated soils.*

Keywords: aluminum, acid soils, magnesium, soil fertility

INTRODUCTION

Lime materials containing calcium (Ca) or magnesium (Mg) alleviate aluminum (Al) toxicity in acidic soils by decreasing the Al saturation of the effective cation exchange capacity (ECEC) and the Al concentration in the soil solution (Kamprath, 1984). Increased Ca and Mg concentrations can also reduce Al activity by increasing soil solution ionic strength (Brady et al., 1993; Wheeler and Edmeades, 1995). In Oxisols and Ultisols where

Received 9 May 2008; accepted 8 October 2008.
Address correspondence to T. Jot Smyth, Department of Soil Science, North Carolina State University, Raleigh, NC 27695-7619, USA. E-mail: jot_smyth@ncsu.edu

soil acidity problems are common, Al^{3+} and H^+ constraints to plant root growth are closely related to soil mineralogical properties. Crystalline and poorly-crystalline Al hydroxide minerals are abundant in these soils (Buol et al., 1997) and provide a source of phytotoxic Al. Trivalent Al (Al^{3+}) is the most toxic species to root growth (Kinraide, 1997) and is present in most soils when soil solution pH values are below 5 (Lindsay, 1979). Hydroxides of Al and iron (Fe) contribute to pH buffer capacity and Al and Fe from minerals are quantitatively more significant than exchangeable Al (Coleman and Thomas, 1964; Volk and Jackson, 1964). Therefore, clay mineralogy is closely related to the ion exchange and pH buffering capacity, which affects Al toxicity in acidic soils.

Recent studies in hydroponics indicate that the ameliorative effect of Ca^{2+} and Mg^{2+} on Al rhizotoxicity can occur even when Al activity in solution remains constant (Lazof and Holland, 1999; Silva et al., 2001a). Silva et al. (2001b) reported that, in contrast with Ca^{2+} additions, beneficial root elongation responses to micromolar Mg^{2+} additions did not coincide with reduced Al activity or increased electrical potential at the surface of the root plasma membranes. These results suggested that Ca affects the reduction of Al activity in solution through electrostatic mechanisms, whereas Mg amelioration of Al rhizotoxicity may involve physiological mechanisms such as organic acid secretion from the root tip and external complexation of Al in solution (Ma, 2005; Matsumoto, 2005).

Many of the studies evaluating the specific effect of Mg^{2+} on Al alleviation of soybean root growth have been conducted in hydroponic systems rather than in the soil environment. Therefore, the effect of Mg^{2+} on alleviation of Al constraints to soybean root elongation is unclear in an acid soil system. The objective of this study was to assess the ameliorative effect of Mg additions on soybean root elongation into acid subsoils of representative Ultisols in the Southeastern US, particularly, the effectiveness of minor additions of Mg in alleviating Al toxicity relative to that of a Ca lime source.

MATERIALS AND METHODS

Greenhouse Experiment

A greenhouse experiment was conducted at North Carolina State University, Raleigh, NC, USA from June to July 2005. A soybean cultivar [*Glycine max* (L.) Merr. cv 'Plant Introduction (PI) 416937'] was chosen because it demonstrated the greatest response in root elongation to increased root-tip citrate amelioration of Al rhizotoxicity among 8 soybean cultivars (Silva et al., 2001d). The plants were grown with a modified vertical-split root system described by Sanzonowicz et al. (1998). A plastic cylinder with 10 cm diameter and 52 cm length was divided into two vertical compartments separated by a root permeable membrane. The surface 12-cm compartment was filled with 1.25 kg of limed and fertilized Wagram soil (loamy, siliceous, thermic, Arenic Kandiuldults). Thus, roots from soybean seedlings in the surface compartment grew in a media with no Al constraint before extending into the underlying compartment containing acid subsoil from three NC Ultisols: Cecil (fine, kaolinitic, thermic Typic Kanhapludults), Creedmoor (fine, mixed, semiactive, thermic Aquic Hapludults) and Norfolk (fine-loamy, kaolinitic, thermic Typic Kandiudults).

Each subsoil was amended with different levels of magnesium chloride ($MgCl_2$) or calcium carbonate ($CaCO_3$). There were three Mg levels consisting of the native equilibrium soil solution concentrations in each soil, 50 μM Mg for Creedmoor and Norfolk, and 100 μM Mg for Cecil (denoted as Control), and $MgCl_2$ additions to achieve 150 and 300 μM Mg (denoted as Mg150, Mg300, respectively) in the soil solutions. Soil solution was extracted using a centrifuge method with 40 hours of equilibration with deionized water (Hashimoto, 2006). After the soil solution was passed through 0.45 μm membrane filter, Mg and other cations were determined by inductively coupled plasma optima emission spectrometry (ICP-OES). The Mg treatment levels were determined based on a previous study demonstrating that up to 1000 μM Mg^{2+} additions improved root elongation of 'PI 416937' cultivar in the presence of Al in hydroponics (Silva et al., 2001a). An additional amendment, lime as $CaCO_3$ added to raise soil pH to a value of 6, was included to compare root elongation response without rhizotoxic Al levels to that of the Mg treatments. Water to achieve 90% container capacity and Mg solutions were added to each soil seven days before transplanting seedlings to allow equilibration of the soil solution ionic composition. Magnesium additions to achieve the desired initial soil solution Mg concentrations, soil solution ionic composition, lime requirement, and soil water container capacity were determined through laboratory studies prior to the greenhouse root-growth experiment (Hashimoto, 2006).

Experimental treatments were arranged in a randomized complete block design with six replicates. Upon harvesting soybean plants after 28 days of growth, three of the six replicates were used to obtain soil samples for physical and chemical analyses, and the other three replicates were used to determine root length after wet-sieve separation from soil. The experimental design was a factorial arrangement of three subsoils and four amendments. Five pre-germinated seedlings were initially transplanted to the surface soil compartment of each cylinder and thinned to two plants after five days. Water content in the surface soil compartment was adjusted daily to 90% container capacity using readings from a time domain reflectometer and a previously calibrated curve with soil water content.

Soil and Plant Analyses

Residual soil water content of the subsurface compartment was determined at harvest for soil samples collected at four depth increments (0–10 cm, 10–20 cm, 20–30 cm, and 30–40 cm). Soil samples were oven dried at 105°C for 48 hours and the water contents were determined by the weight difference. Plant water uptake by roots growing in the subsurface compartment was calculated as the difference in soil water content between the initial container capacity at planting and the remaining soil water at harvest.

Soil physical and chemical characteristics were determined on air-dried samples ground to pass a 2 mm sieve. Soil texture was determined by a hydrometer (Gee and Bauder, 1986), and organic matter content was determined by a loss-on-ignition method (Nelson and Sommers, 1996). Soil pH was measured at a 1:2.5 soil-water ratio. Exchangeable Al, Ca, and Mg were extracted with 1M potassium chloride (KCl) solution, and exchangeable potassium (K) was extracted with the Mehlich 3 solution (Mehlich, 1984) at a 1:10 soil-solution ratio. All exchangeable cations were determined by atomic absorption spectrometry. Acid ammonium-oxalate extractable Al and Fe (dry soil weight: 0.8 g) and citrate-bicarbonate-dithionite (CBD) extractable Al and Fe were determined for all subsoils with three replicates (dry soil weight: 1.0 g for Cecil and 3.0 g for Creedmoor and Norfolk) based on the procedure of Jackson et al. (1986). All extracts were filtered through a 0.45 μm membrane and analyzed for Al and Fe by atomic absorption spectrometry.

Soybean roots were separated at harvest from soil in the surface and subsurface compartments by washing through a 0.5 mm sieve. Root length was determined by an edge discrimination method (Pan and Bolton, 1991) using a desktop scanner preset to a resolution of 29.5 dots cm^{-1}. Relative root length among treatments for each subsoil was calculated as a percent of root length for the limed treatment. Aboveground tissues of plants harvested at 28 days were dried at 60°C in a forced-draft oven, and the dry weights were measured. Dried plant material (0.5 g) was digested with 3 mL of 6 M hydrochloric acid (HCl) and 2 mL of concentrated nitric acid (HNO_3) in a hot water bath. After digestion, the solution was filtered, diluted with deionized water, and analyzed for Mg by atomic absorption spectrometry.

Citrate Adsorption and Biodegradation Experiment

A batch experiment was conducted to assess the possible fates (adsorption and biodegradation) of citrate in the acid subsoils under microbial-active and inactive conditions. The adsorption methods and procedures used were a modification from Hutchison and Hesterberg (2004). Sieved soil samples (3.0 g) collected from the subsurface compartment of the

Control treatments were weighed into tared 40 mL polycarbonate centrifuge tubes. After the addition of 10 mM KCl solution with or without 0.6 mM sodium (Na) azide as a soil sterilizer, the samples were incubated at 25°C for 24 hours. Potassium citrate monohydrate solution, adjusted to pH 4.5 by adding 10 mM HCl, was added to each duplicate tube yielding final citrate concentrations between 0 and 1111 μM. After the samples were equilibrated for 12 hours by shaking, each suspension sample was adjusted to pH 4.5 with 10 mM HCl or potassium hydroxide (KOH), and shaking continued for another 12 hours. After the 24-hour equilibration period, the samples were centrifuged at 16000 rpm for 10 minutes. Supernatant solutions passed though a 0.2 μm membrane filter were analyzed for dissolved citrate concentration by ion chromatography. Concentration-dependent adsorption isotherms of citrate were modeled for each subsoil by the Freundlich equation:

$$x = ac^n$$

where x is the amount of adsorbed citrate (μmol kg^{-1}), c is the equilibrium solution citrate concentration (mM), and a and n are constants (Fitter and Sutton, 1975).

Statistical Analysis

For each subsoil, analysis of variance for a full factorial treatment arrangement was performed for exchangeable cation concentrations, root length, plant dry weight and root water uptake followed by mean separation with Fisher's LSD by SAS, Version 9.1 (SAS Institute, Cary, NC, USA). When no interactions were found between subsoils and amendments, as with plant dry weight, main effects of each treatment were compared. Analysis of variance was also used for mean separation of citrate concentration/adsorption in the citrate adsorption and biodegradation experiment. The correlation procedure was performed to compute the correlation between subsoil and topsoil root lengths. The regression procedure was used to determine the regression curve of plant water uptake as a function of relative root length. For assessment of diagnostic indices among the subsoils, the subsoil root length was expressed as a percentage of the root length in the lime treatment of the same subsoil (relative root length). Regression equations were determined on the relative root length expressed as a function of soil exchangeable cations.

RESULTS

Soil Characterization

All subsoils without Mg or lime additions were characterized as acidic with pH values below 4.6 (Table 1). The Creedmoor subsoil had the highest

TABLE 1 Exchangeable cations, Al saturation and pH of subsoils amended with different amounts of Mg and lime after harvesting soybean

		Exchangeable Cation					Al	
Soil	Treatment	Ca	Mg	K	Al	ECEC	Saturation	pH
				$cmol_c\ kg^{-1}$			%	
Cecil	Control	0.52	0.46	0.28	0.46	1.72	27	4.4
	Mg150	0.51	0.45	0.28	0.46	1.70	27	4.5
	Mg300	0.49	0.52	0.25	0.46	1.72	27	4.5
	Lime	2.32	0.38	0.22	0.05	2.97	2	5.9
	Mean	0.96	0.45	0.25	0.35	2.03	20	4.8
Creedmoor	Control	0.08	0.08	0.09	1.22	1.47	83	4.3
	Mg150	0.08	0.08	0.09	1.14	1.39	82	4.4
	Mg300	0.07	0.09	0.07	1.15	1.38	83	4.4
	Lime	3.18	0.07	0.08	0.18	3.51	5	5.6
	Mean	0.85	0.08	0.08	0.92	1.97	61	4.7
Norfolk	Control	0.07	0.01	0.06	0.22	0.36	61	4.6
	Mg150	0.07	0.02	0.05	0.21	0.35	60	4.6
	Mg300	0.07	0.02	0.05	0.19	0.33	58	4.8
	Lime	0.56	0.01	0.04	0.04	0.65	6	5.7
	Mean	0.19	0.02	0.05	0.16	0.42	45	4.9
				Treatment Means				
	Control	0.22	0.20	0.14	0.63	1.19	56	4.4
	Mg150	0.22	0.19	0.13	0.60	1.16	55	4.6
	Mg300	0.21	0.22	0.12	0.59	1.16	54	4.5
	Lime	2.01	0.16	0.11	0.08	2.38	4	5.7
$LSD_{0.05}$								
Soil		0.03	0.01	0.01	0.03	0.06	1	0.04
Treatment		0.03	0.02	0.01	0.04	0.07	1	0.05
Soil × Treatment		0.07	0.04	0.02	0.08	0.12	2	0.10

$LSD_{0.05}$: Fisher's least significant difference (LSD) values of means determined at $P = 0.05$.
Control, without Mg and lime amendment; Mg150 and Mg300, adjusted soil solution Mg concentration with 0.15 and 0.3 mM, respectively; Lime: amended with $CaCO_3$.

exchangeable Al and Al saturation values, suggesting the strongest acidity constraints to root elongation among the three subsoils. Exchangeable Ca, Mg, and K were low (< 0.1 $cmol_c$ kg^{-1}) in the Creedmoor and Norfolk subsoils as compared to the Cecil subsoil. The Creedmoor and Norfolk subsoils had a sandy loam texture with 1.9 and 1.5% OM, respectively. The Cecil subsoil had a clay texture with 11.4% OM.

Exchangeable Mg of Creedmoor and Norfolk subsoils was not different among the treatments ($P > 0.05$), whereas the Mg300 treatment of the Cecil subsoil presented a higher value than other treatments ($P < 0.05$). There were no differences in exchangeable Al among Mg-amended treatments (Mg150 and 300) as compared to the treatment without added Mg or lime (Control) for all subsoils ($P > 0.05$). The absence of changes in exchangeable Al and Mg between treatments with or without Mg amendments indicates that additions of Mg did not influence the exchanger-phase composition with respect to these cations. Although exchangeable Mg tended to increase slightly with 150 and 300 μM Mg in all subsoils, there were no measurable changes in Al saturation as compared to the Control treatments ($P > 0.05$). Liming of all subsoils reduced Al saturation, raised exchangeable Ca and increased pH values above 5.5, where concentrations of the rhizotoxic Al^{3+} species in the soil solution would be nil (Kamprath and Smyth, 2005).

The concentrations of CBD-extractable Fe were typically higher in the Cecil subsoil than other subsoils (Table 2), resulting from more clay and abundant Fe-oxide minerals in the Cecil subsoil. The greater oxalate- to CBD-extractable Fe ratio (0.30) for the Creedmoor subsoil as compared to other subsoils indicated a greater proportion of poorly crystalline Fe-oxides in the Creedmoor subsoil (Schwertmann, 1993). The concentrations of CBD-extractable Al were typically higher in the Cecil than in other subsoils, corresponding to the concentrations of CBD-extractable Fe.

TABLE 2 Crystalline and poorly-crystalline Al and Fe in the subsoils used for the soybean root growth study

	Crystalline and poorly crystalline Fe and Al				
Soil	Fe-ox	Fe-CBD	Fe_o/Fe_c	Al-ox	Al-CBD
	mg kg^{-1}			mg kg^{-1}	
Cecil	59	1853	0.03	105	303
Creedmoor	33	112	0.30	41	61
Norfolk	14	113	0.12	31	61

Fe-ox, Al-ox: oxalate extractable Fe and Al, respectively.
Fe-CBD, Al-CBD: citrate-bicarbonate-dithionite extractable Fe and Al, respectively.
Fe_o/Fe_c: ratio of oxalate extractable Fe to CBD extractable Fe.

Root Growth

Subsoil root length for the treatments without added Mg or lime (Control) decreased in the order of Cecil followed by Norfolk then Creedmoor subsoils (P < 0.05), and corresponded to the increasing order of percent Al saturation (Figure 1). There were interaction effects between subsoils and amendments (P < 0.05). Limed Cecil and Creedmoor subsoils had 1.3-fold and 4.5-fold more subsurface root length relative to the Control treatments. Root elongation response to lime increased with initial subsoil exchangeable Al levels. However, the subsoil root length of the limed treatment in the Norfolk subsoil was not different from the other treatments (P > 0.05). There were no statistical differences in subsoil root length for the Mg150 and Mg300 treatments as compared to the Control treatments in any of the subsoils (P > 0.05).

Relative root length among subsoil treatments was related to soil exchangeable Al and Al saturation (Figure 2), consistent with Al being a primary factor inhibiting root growth in these acid subsoils. Aluminum saturation is often used as an index to estimate Al rhizotoxicity of plants in acid soils (Kamprath, 1984). For example, soybean growth is generally restricted when Al saturation exceeds 15% (Osmond et al., 2002). The lime treatment in all subsoils reduced the Al saturation below 6%, whereas Al saturation of the Mg150 and Mg300 treatments was similar to that of Control treatments. Since exchangeable Mg in all subsoils was virtually unchanged with the Mg

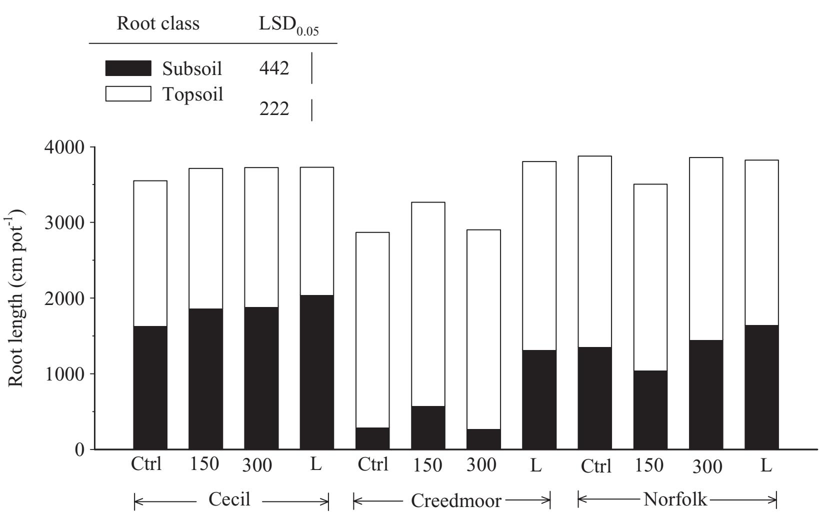

FIGURE 1 Root length in the surface compartment, and the subsurface compartment (Cecil, Creedmoor and Norfolk subsoils) with either native soil Mg levels (Ctrl), treated with $MgCl_2$ solution to achieve 150 and 300 μM Mg in the soil solution (150 and 300, respectively), or limed with $CaCO_3$ to neutralize exchangeable Al (L). The least significant difference (LSD) for each mean indicated as vertical bars represents the soil x amendment interaction which was significant at P = 0.05.

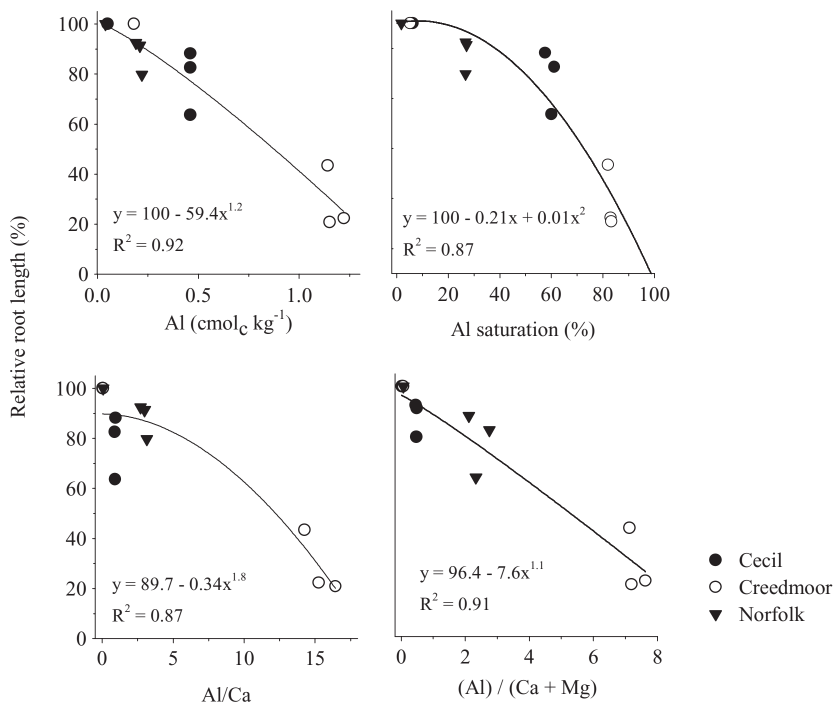

FIGURE 2 Observed (symbols) and predicted (lines) relative root length of soybean grown in three subsoils treated with different Mg concentrations or limed to a pH value of 6.0, expressed as a function of parameters associated with soil exchangeable cations. Relative root length is expressed as a percentage of the root length in the lime treatment of each soil (100% = lime treatment).

additions (Table 1), Al saturation values remained at rhizotoxic levels, leading to less root length in the Mg treatments than that of the limed treatments. Although our study found a strong relationship between exchangeable Al and relative root length, some previous studies conducted in acid soils from Australia reported a poor relationship between these parameters (Bruce et al., 1988; Menzies et al., 1994). In addition to exchangeable Al, over 87% of the variability associated with relative root length was explained by the exchangeable concentration ratios of Al/Ca and Al/(Ca + Mg) (Figure 2). This result indicates that exchangeable Ca, as well as Al, may be a factor determining root growth in the acid subsoils examined here. Bruce et al. (1988) proposed that exchangeable Ca would be an important parameter to predict relative root length in acid soils with low Ca concentrations as found in the subsoils used in our study. Regressions of exchangeable Mg or the ratio of Al/Mg on relative root length gave low R^2 values of < 0.27, because adding Mg did not increase exchangeable Mg. This result supports the previous observation on the absence of improvement in root growth in the Mg150 and 300 treatments as compared to the Control treatments for all subsoils ($P > 0.05$).

There were significant main effects ($P < 0.05$) of soil and amendment on root length of the surface soil compartment. Because no interactions were found between soil and amendment, averaged surface compartment root length across these treatments (soil or amendment) is used for the following discussion. Surface soil root length of lime treatments was less than for other treatments ($P < 0.05$). When compared to the lime treatment, surface soil root length increased by approximately 170 cm in the Mg150 treatment, 190 cm in the Mg300 treatment, and 200 cm in the Control treatment. Reduced surface soil root length in the lime treatment indicates a preferential root proliferation into the limed subsoil. This tendency was also explained by the evidence that root length was negatively correlated ($r = 0.82$; $P < 0.01$) between the surface and subsurface compartments.

Root length in the surface compartment averaged across all treatments decreased in the order of Creedmoor (2544 cm) followed by Norfolk (2384 cm) and Cecil (1799 cm) ($P < 0.05$). More specifically, the subsoil with the largest acidity constraints (high Al saturation and low pH value) had the least root length in the subsurface compartment and the greatest root length in the surface compartment. This inverse relation between root length in surface and subsurface compartments and soil acidity constraints corresponded to findings by Smyth and Cassel (1995) for a field trial, wherein over 70% of corn root growth occurred in the top 5 cm of an Oxisol profile when no lime was applied; lime applications of 2 and 4 t ha^{-1}, however, reduced the root growth in the surface soil by 45% and increased the root distribution to the subsoil layers.

Plant Dry Weight

Mean values of aboveground plant dry weight were similar among the subsoils, but there were differences among the Mg and lime treatments to the subsurface compartment ($P < 0.01$). Since there were no subsoil $\times$ amendment interactions, the data were averaged across the subsoil treatments (data not shown). The plant dry weight of the limed treatment (1.54 g) had the highest value ($P < 0.05$) and decreased to 91% (1.41 g) in the Mg150 treatment, 89% (1.38 g) in the Mg300 treatment, and 85% (1.31 g) in the Control treatments. There were no differences in plant dry weight among the Control, Mg150 and Mg300 treatments ($P > 0.05$), which corresponded to the trends found in mean root length among the subsurface lime and Mg treatments.

Plant Water Uptake from the Subsurface Compartment

Plant water uptake from the subsurface compartment differed among the subsoils, treatments and subsoil by treatment interactions ($P < 0.01$; data not shown). When averaged across treatments, more water was taken

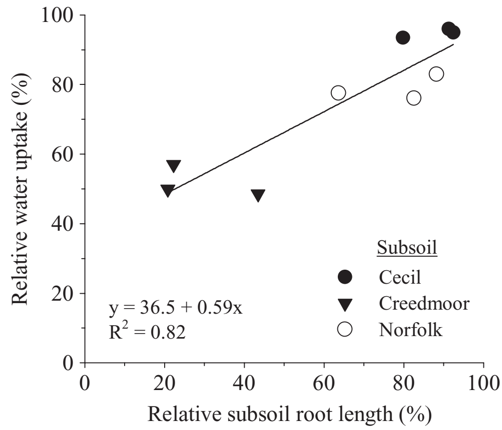

FIGURE 3 Observed (symbols) and predicted (line) relative plant water uptake from the subsoil as a function of relative root length in the subsurface compartment. Both relative values are expressed as % of limed treatments.

up from the Cecil subsoil (360 g; $P < 0.01$), but there was no difference between the Creedmoor and Norfolk subsoils (225 g each). Liming of the Creedmoor and Norfolk subsoils increased water uptake as compared to the Mg treatments (Control, Mg150 and Mg300; $P < 0.05$). There were no differences in plant water uptake among the Mg treatments for the Creedmoor and Norfolk subsoils ($P > 0.05$), which matches the lack of differences in subsoil root length among the Mg treatments for these soils (Figure 1). When compared to the lime treatment, averaged plant water uptake decreased by approximately 80 g in the Control, 86 g in the Mg150, and 81 g in the Mg300 treatments for all subsoils. Within the Cecil subsoil, there were no differences in plant water uptake among the treatments ($P > 0.05$). Relative water uptake expressed as a percent of the limed treatment was predicted as a function of relative subsoil root length (Figure 3). Plant water uptake from the subsoil increased linearly with increasing relative root length among the subsurface compartment treatments. Increased root length improved the accessibility to and plant uptake of the available water in the subsurface compartment. Relative water uptake increased in the order of Creedmoor, Norfolk and Cecil subsoils, which corresponded to the decreasing order of Al constraints (e.g., Al saturation and exchangeable Al levels in Figure 2) among subsoils.

Magnesium Accumulation in Aboveground Plant Tissues

Magnesium accumulation in plant tissues differed among the treatments and subsoils ($P < 0.05$; data not shown). Plant Mg accumulation in the Cecil subsoil followed the decreasing order of lime and similar values for the Mg150 and Mg300 treatments. In the Creedmoor subsoil, Mg accumulation in the lime treatment was superior to that of the Mg treatments. When compared to the Control treatment with the lowest Mg values within each subsoil, plant Mg accumulation of the lime treatments increased by 1.2-fold in the Cecil and 1.5-fold in the Creedmoor subsoils. Since the treatment with $CaCO_3$ provided no additional Mg supply, increased plant Mg accumulation upon liming was associated with the improved root growth in the Al-neutralized subsoil. Baligar et al. (1993) found that Mg concentrations in sorghum tissues increased from 0.01 to 0.23 mmol plant^{-1} when Al saturation of an acid soil was reduced from 64 to 2%. In contrast to the Cecil and Creedmoor subsoils, the largest plant Mg accumulation for the Norfolk subsoil value was found in the Mg300 treatment ($P < 0.05$). Because there were no apparent differences in the subsoil root length among the Mg treatments, increased plant Mg accumulation in the Mg300 treatment was likely related to the addition of Mg in the subsoil.

Increasing Mg levels resulted in increased plant Mg accumulation, indicating that indeed the Mg in the soil solution was available to roots and absorbed in tissues. Silva et al. (2001c) demonstrated that increased additions of micromolar concentrations of Mg^{2+} to hydroponic solutions enhance Al tolerance of soybean by increasing citrate secretion from roots and external complexation of toxic Al species in solution. More recently, Yang et al. (2007) reported enhanced root growth of rice bean [*Vigna umbellata* (Thumb.) Ohwi & Ohashi] was accompanied by citrate efflux upon micromolar Mg^{2+} additions in an Al stressed condition. However, our result demonstrated that root growth in all subsoils was not improved even when the soil solution had micromolar levels of plant-available Mg sufficient to induce root citrate production and secretion in hydroponic systems.

Citrate Adsorption and Biodegradation in Subsoils

Unlike hydroponic systems, organic acids secreted from the root to the soil solution can potentially be sorbed or biodegraded. Adsorption isotherms were determined to characterize the citrate adsorption capacity of subsoils (Figure 4a). Adsorption isotherms for all subsoils were adequately described by the Freundlich equation (Table 3). The amount of citrate adsorption among subsoils generally followed the order of Cecil > Norfolk > Creed-

TABLE 3 Citrate adsorption isotherms and regression coefficients (R^2) predicted by the Freundlich equation for subsoils as shown in Figure 4(a)

Subsoil	Freundlich equation	R^2
Cecil	$x = 18.6c^{0.3}$	0.91
Creedmoor	$x = 9.1c^{3.3}$	0.93
Norfolk	$x = 15.1c^{1.4}$	0.96

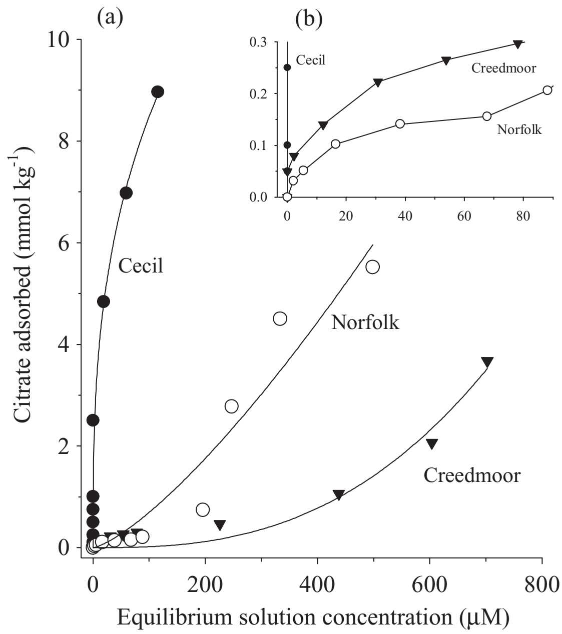

FIGURE 4 Concentration-dependent adsorption isotherm of citrate for the Cecil, Creedmoor and Norfolk subsoils (Control treatment) determined at a solution pH value of 4.5 with microbial inhibition by Na-azide additions. Citrate adsorption isotherms of all subsoils were predicted by the Freundlich equation (lines). A magnified figure shows the trend of citrate sorbed to the subsoil in the scale up to 90 μM citrate solution concentration.

moor. Across the concentration range used in this study, the Cecil subsoil had the highest citrate adsorption when the citrate addition exceeded 27 μM ($P < 0.05$). The Cecil subsoil had the highest affinity for citrate adsorption among the subsoils with 99% adsorption observed throughout the dissolved citrate concentration range. The high adsorption of citrate for the Cecil subsoil resulted from its clayey texture with abundant crystalline and poorly-crystalline Al and Fe hydroxides (Table 2).

The amount of citrate adsorption on the Norfolk subsoil was greater than that of the Creedmoor subsoil when the citrate addition exceeded 270 μM ($P < 0.05$), despite subsoil similarities in clay, OM contents, and amount of crystalline Al and Fe and poorly-crystalline Al. The different characteristics in citrate adsorption between the Creedmoor and Norfolk subsoils could be related to differences in the predominant clay minerals (montmorillonitic for Creedmoor and kaolinitic for Norfolk). With an acid pH (4.5), kaolinite possesses more positive net charge due to a higher point of zero-charge value than montmorillonite (Stumm and Morgan, 1981), thus leading to more citrate adsorption for the Norfolk subsoil. At low solution citrate concentrations of 11 to 111 μM, however, there was more citrate adsorption by the Creedmoor than by the Norfolk subsoil ($P < 0.05$; Figure 4b). This result indicates that poorly-crystalline Al and Fe hydroxides, which were more abundant in the Creedmoor subsoil would be a primary adsorption site for citrate (Jones and Edwards, 1998; Jones et al., 1996) and these clay minerals may have a significant role in controlling the citrate concentration at the low solution concentrations. A high adsorption capacity of Fe hydroxide for citrate, 650 mmol kg^{-1} at a pH value of 5.0, was reported by Jones and Brassington (1998). Thus, we might expect that citrate secreted from roots to soils, which ranges from nano- to micromolar levels (Jones, 1998), would initially be adsorbed onto poorly-crystalline Al and Fe.

Biodegradation can be an alternative fate of citrate secreted from the root into the soil. Because the microbial activity in an acidic Al toxic soil is generally inhibited (Amonette et al., 2003; Illmer et al., 2003), the higher exchangeable Al levels of the Creedmoor subsoil, relative to the Norfolk subsoil, could result in less microbial degradation of citrate in the former soil. The citrate concentration in solution for a given input concentration under the microbial inhibited condition (with Na-azide addition) was higher than that under the microbial active condition (without Na-azide addition) when the citrate concentration exceeded 30 μM for the Creedmoor and 10 μM for the Norfolk subsoils ($P < 0.05$; Figure 5). The difference in solution citrate concentrations between treatments with and without Na-azide additions was attributed to the amount of microbially-degraded citrate. The amount of microbially-degraded citrate across the entire solution concentration range are shown as shaded areas, in Figure 5, and are greater in the Norfolk (3510 μM) than the Creedmoor (2220 μM) subsoils. In the Cecil subsoil, all citrate was absorbed from solution both with and without Na-azide additions, indicating the predominance of adsorption rather than biological degradation as the potential fate of citrate. Adsorption characteristics of the Cecil subsoil at low solution concentrations would suggest that root-secreted citrate would be unavailable to complex rhizotoxic Al^{3+} in the soil solution.

DISCUSSION

Our study demonstrated that liming of acidic subsoils in the subsurface compartment generally improved root growth, dry matter production and plant water uptake. Additions of lime to the acid subsoils reduced exchangeable Al and Al saturation and enhanced root and plant growth. Contrarily, these growth parameter responses to the Mg treatments were less than for the lime treatments (Figure 1). Additions of Mg to achieve micromolar levels in the soil solution did not influence the exchanger-phase composition (i.e., exchangeable Al and Al saturation), indicating that Al toxicity remained in these soils (Table 1). A mechanistic description of cation amelioration of Al rhizotoxicity has been proposed by Kinraide (1998) who comprehensively elucidated electrical potential and Al^{3+} activity at the plasma membrane of the root surface by employing a Gouy-Chapman-Stern model. Based on his

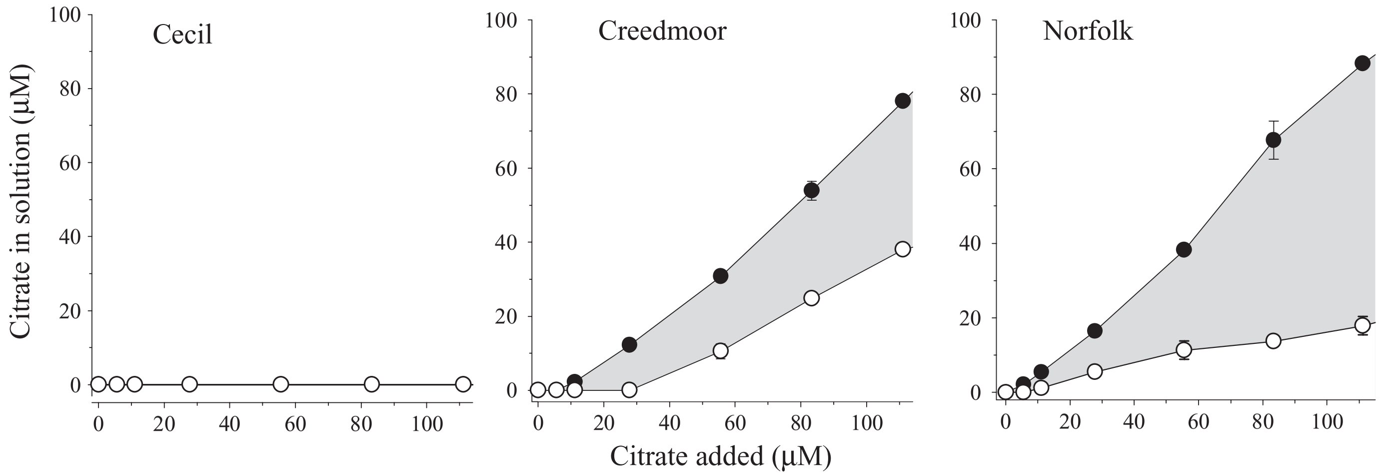

FIGURE 5 Citrate concentration in solution after 24 hour equilibrium as influenced by increased concentrations of citrate additions with (filled symbols) or without (open symbols) Na-azide additions for the Cecil, Creedmoor and Norfolk subsoils (Control treatment) at a solution pH value of 4.5. Data for Na-azide additions (filled symbols) for the Cecil subsoil were not presented because these are equal to the values that were obtained in the treatment without Na-azide additions. The shaded area in the plot (Creedmoor and Norfolk) indicates the amount of biodegraded citrate.

theory, ameliorative effects of cations at millimolar concentrations could be explained in terms of increased electrical potential and subsequent reductions of Al^{3+} activity at the root plasma membrane surface.

Silva et al. (2001b) reported that Ca and Mg were equally effective ions for alleviation of Al rhizotoxicity in soybean when added in millimolar concentrations. In the micromolar range, however, this electrostatic effect for alleviating Al toxicity seems to be less valid for Ca than for Mg. For example, root growth response of soybean (Silva et al., 2001b) and rice bean (Yang et al., 2007) to Al toxicity was not improved by micromolar Ca additions that did not affect electrical potential of root plasma membrane and Al^{3+} activity in solution. An ameliorative effect of micromolar Mg on Al rhizotoxicity has also been reported in other plant species including sorghum (Tan et al., 1992) and rice (Watanabe and Okada, 2005), but not wheat (Kinraide, 1998), indicating that ameliorative responses to micromolar Mg has specificity to plant species. Evidence for the role of Mg indicated a physiological ameliorative effect that involves increased production and exudation of citrate in the root tips, leading to external complexation of toxic Al species in solution (Silva et al., 2001d; Yang et al., 2000). Kinraide et al. (2004) suggested that the ameliorative mechanism of Al toxicity by Mg at a low concentration is beyond pure electrostatic effects and involves physiological effects that depend on plant genotype. For our investigations with acid soil systems, however, the addition of Mg to achieve micromolar soil solution concentrations did not improve root growth in the subsoils (Figure 1). These findings may suggest that the amelioration of Al rhizotoxicity by inducing a physiological defense mechanism such as citrate exudation from the root tip are less pronounced in acid soils than in hydroponic systems.

Our results suggest the possibility that the ameliorative mechanism induced by micromolar Mg is valid when the soil has certain levels of Ca available for root growth. Silva et al. (2001a) suggested that there are absolute Ca levels in solution to express the physiological protective effect (i.e., citrate production) of Mg on soybean cultivars since Al inhibition of root elongation failed to be alleviated in solution below 300 μM Ca concentration, regardless of increased Mg supply. Similar protective effects of Mg on Al rhizotoxicity were confirmed under a sufficient Ca supply (> 500 mM Ca^{2+}) for rice been (Yang et al., 2007) and soybean (Yang et al., 2001). The Ca^{2+} in a rooting medium is essential for root elongation, even in the absence of added toxicants. In acid soils expressing Al toxicity, one of the most common symptoms of toxicity is Ca deficiency (Rengel, 1992), leading to the proposal of Ca^{2+} channel blockage as the primary lesion due to Al toxicity to plant roots (Rengel, 1992; Rengel et al., 1995). Calcium deficiency also enhances plant root vulnerability to increased H^+ conditions because Ca has an ameliorative effect of H^+ toxicity (Sanzonowicz et al., 1998). Contrary to previous hydroponic studies, the present study conducted in acid soils had low Ca concentrations as indicated by Ca concentration in the soil exchangeable phase (Table 1). Our study demonstrated little improvement of root growth particularly in the Creedmoor and Norfolk soils with Mg150 and 300 treatments (Figure 1) where Ca concentrations in the soil solution were below 350 μM (Hashimoto, 2006). Three acid subsoils used herein were Ultisols characterized by highly weathered minerals with low exchangeable Ca (Buol et al., 1997). Because all the subsoils investigated had low native soil solution Ca concentrations, the possibility of restricted root growth through a concurrent Ca deficiency in the Mg-treated subsoils as compared to the limed subsoils cannot be ruled out (Hashimoto, 2006). It remained unknown whether citrate secretion induced by Mg additions occurs in acid soils with a low native Ca concentration.

Another important aspect of absent root growth response in the Mg-treated subsoils could be associated with soil mineralogical and biological effects on the availability of root-secreted citrate. Our study demonstrated citrate concentrations extracted with a weak HCl solution (method referred to (Kirk et al., 1999) from all soils adhering roots after plant harvest were below detection limit (data not shown). This indicates that secreted citrate from the root to soil solution, if it is induced by micromolar Mg additions, suffered from sorption and biodegradation, preventing citrate from forming non-toxic Al-citrate complexes. Citrate adsorption could be a primary factor lowering the citrate concentration in the soil solution as evidenced by negligible amounts of citrate detected in the solution isotherms for the Cecil subsoil (Figure 4). In soils with low clay content such as the Creedmoor and Norfolk subsoils, degradation and mineral-dependent sorption apparently play important roles in controlling soil solution citrate concentration. Van Hees et al. (2002) found that oxalate mineralization in an organic soil occurred at approximately 4-fold the rate of that in a soil with low organic content, indicating that the organic soil with high microbial activity can generally increase the decomposition rate of an organic acid in the soil solution. Chemisorption of citrate by Al and Fe hydroxides has shown that organic acid sorption is primarily pH dependent, with increasing sorption capacity in an acidic soil (Jones and Brassington, 1998; Karltum, 1998). Soils dominated by kaolinite often showed a higher adsorption capacity for citrate and dissolved organic carbon than those dominated with 2:1 clay minerals including mica, illite and montmorillonite (Jones and Edwards, 1998; Kahle et al., 2004; Lackovic et al., 2003). The difference in citrate adsorption between these minerals increased when the solution contained a high equilibrium citrate concentration (Jones and Edwards, 1998; Lackovic et al., 2003), and similar adsorption characteristics were found in our study. Citrate adsorption capacity of the subsoils did not correlate with ECEC and exchangeable Al, which agreed with the study conducted by Jones and Brassington (1998), supporting the evidence that these acid soils were dominated by highly weathered clay minerals with little contribution for soil's cation exchange capacity. Acid soils dominated with Al/Fe hydroxides and kaolinite-dominated clay fractions are characterized as Ultisols and Oxisols that are the major soil series found in tropical and subtropical climatic areas (Buol et al., 1997). As for the efficiency of root physiological defense to Al mediated by citrate and likely other organic acids, therefore, sorption and biodegradation effects in the acid soils potentially reduce the availability of root-exuded citrate to form non-toxic Al complexes in the soil-root interface. However, it should be noted that the batch experiment approach used herein may overestimate the soil's citrate sorption and biodegradation capacity because secreted organic acids may not directly interact with soil minerals by the protective effect of rhizodepositions (Gobran et al., 2005). Although the relative trend of potential citrate fate among the mineralogically different acid soils was illustrated by this approach, further investigation is needed to understand the fate of organic acids secreted into the root elongation front where organic acids are highly concentrated.

Our study assessing the ameliorative effect of Mg on Al toxicity in acid soils provides implications to current hydroponic methods commonly employed for screening Al tolerant species. Hydroponic procedures have been widely used for screening and ranking Al tolerant genotypes for soybean and other plant species because of their advantages in cost- and time-effectiveness. However, the genotype rankings for Al sensitivity determined by hydroponics were found to be inconstant among the procedures used and were most likely influenced by solution compositions (Lazof and Holland, 1999; Spehar, 1994). Previous studies reported vulnerability of hydroponic methods, attesting that Al tolerant cultivars characterized by the hydroponic experiment did not always demonstrate a better growth in acid soils (Bushamuka and Zobel, 1998; Ritchey and Carter, 1993). Based on our present study, these inconsistent results in screening of Al tolerant species between hydroponics and soils may result from the fact that hydroponics cannot completely reflect the soil physicochemical and biological properties (i.e. sorption and microbial degradation) influencing the fate of organic acids in the soil. Moreover, poor nutritional conditions of common acid soils (e.g., Ca deficiency), which may be a potential confounding of physiochemical functions affecting Al tolerance, are difficult to reproduce in hydroponic systems. For a better understanding of interactions among Al/Mg and Ca deficiency on citrate secretion by soybean cultivars, further studies employing acid soils should be linked with current hydroponic-based studies.

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